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Microwave-Assisted Synthesis, Characterization and Cytotoxic Studies of Novel Estrogen Receptor α Ligands towards Human Breast Cancer Cells

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Abstract: A new, simple, and microwave-assisted, solution-phase T3P®-DMSO mediated method for the preparation of a novel class of estrogen receptor alpha (ERα) ligands based on the 2-phenylquinoline scaffold was developed. Furthermore, the novel ERα ligands were tested for their bioactivity against ERα-positive and ERα-negative cell lines. The ligand (entry 4), with amine and nitro group substitution at C4 position, displayed significant cytotoxicity against MCF-7 and HepG2 cells with an IC_{50} value of 6 and 11 μM, respectively. On the other hand, ERα-negative cells displayed resistance to quinolines induced cytotoxicity with an IC_{50} value >100 Mm and they does not induce cytotoxicity in normal breast epithelial cells. Molecular docking analyses suggest a consistent binding mode for these ERα ligands in the ligand binding domain of the human ERα and predict the ligands to occupy the hydrophobic cavity in a similar fashion as estradiol or GW2368.

INTRODUCTION

Breast cancer is a leading cancer in women worldwide and contributing to second cause of lethality after lung cancer.[1, 2] Most breast cancers are associated with interaction of estrogen receptors (ER) in the breast epithelial cells to estrogen. The physiological action of estrogen is induced via two types of estrogen receptors namely ERα and ERβ.[3] Research in the previous decade revealed that more than 70% of breast cancers are due to ERα dependent epithelial cell proliferation. The role of ERβ is not clear in initiation and progression of breast cancer.[4] ERα belongs to nuclear receptor superfamily which regulates the transcription of genes involved in proliferation, anti-apoptosis, metastasis and immunosurveillance.[5-7] The binding of 17β-estradiol to ERα induces the receptor dimerization and facilitates binding of the ligand-receptor complex to the promoter of target genes.[8] Also, ER-dependent pathways regulate the synthesis and distribution of glycosaminoglycans in cancer cells.[9] Several small molecule ERα antagonists including Tamoxifen, Raloxifene and Fulvestrant have been implemented in the treatment of breast cancer.[10, 11] Benzisoxazole tethered azoles have known to be the better ER ligands.[12-14] Therefore, probing small molecules against ERα is considered to be the most attractive therapeutic target to treat breast cancer.[15]

Quinoline derivatives are the pharmacologically important heterocycles which have been studied extensively for their anticancer properties. Multiple reports have demonstrated the preparation of quinolines using strong base like tertiary butoxide.[16, 17] We previous reported the anti-cancer effect of various heterocyclic compounds [18-20] and recently reported the solution phase synthesis of 2-amino-chromene-3-carbonitriles from alcohols, malanonitrile and phenols.[21] Using a similar strategy, herein, we report a simple and efficient method for the preparation of T3P®-DMSO mediated 2-phenylquinoline derivatives using amino alcohols and acetophenones under microwave irradiation and evaluated for their cytotoxicity. The newly developed method was generalized using variety of aromatic 2-amino alcohols

and different substituted acetophenones to obtain **1-10** molecules (**Scheme 2; Table 1**). Our docking analysis validated the interaction of quinoline derivatives with the estrogen receptor in the similar fashion as estradiol to induce its anticancer effect.

Table 1. The physical characterization of new quinoline-based ERα ligands, whose core scaffold has different substitutions at R^1 to R^6 positions.

Entry	R^1	R^2	R^3	R^4	R^5	R^6	Y	mp (^{0}C)
1	H	H	OH	H	H	H	85	52
2	CH_3	H	Br	H	H	H	89	50
3	H	H	Cl	H	Cl	H	92	70
4	H	Cl	H	H	NH_2	H	95	60
5	CH_3	H	Cl	H	Cl	H	90	59
6	H	H	H	H	Br	H	88	63
7	CH_3	H	H	H	H	H	87	54
8	CH_3	H	H	H	NH_2	H	91	67
9	CH_3	H	OH	H	H	OH	89	54
10	CH_3	H	H	NO_2	H	H	93	64

Additionally, all the new molecules were characterized completely using IR, ^{1}H NMR, ^{13}C NMR, and LC-MS spectral analysis (please refer supplementary data).

Further, the library of ERα ligands was tested for its cytotoxicity against ERα-positive, ERα-negative cancer cells and their counterpart non-diseased breast epithelial cells as described previously and detailed methodology is provided in supplementary information.[22, 23] The results are summarized in Table 2.

Table 2. Cytotoxicity data for the new ERα ligands IC_{50} against human cancer cells.

Entry	ERα-positive cells		ERα-negative cells		Breast epithelial cells
	MCF-7 (μM)	HepG2 (μM)	MDA-MB-231 (μM)	BT549 (μM)	MCF-10A (μM)
1	>50	>50	NT	NT	NT
2	>50	>50	NT	NT	NT
3	25	31.6	> 100	> 100	> 100
4	6	11	> 100	> 100	> 100
5	>50	>50	NT	NT	NT
6	12	9	> 100	> 100	60.3
7	>50	>50	NT	NT	NT
8	34	41	NT	NT	NT
9	28	19	NT	NT	NT
10	23	37	> 100	> 100	> 100

*NT – Not tested

Among the tested compounds **4**, **6**, and **10** significantly inhibited the proliferation of ERα-positive cells. Further, compounds (**4, 6,** and **10**) with high cytotoxicity were tested against ERα-negative cells. All the ERα-negative cells were resistance to the lead compounds with IC_{50} values more than 100 μM. However, the shortlisted three molecules did not induce cytotoxicity on MCF-10A cells up to 72 h at 100 μM. These results indicate that the newly synthesized ERα ligands are selectively cytotoxic against ERα expressing cancer cells and does not interfere with viability of their counterpart.

Additionally, the structural models for molecular interactions between the newer ERα ligands and the human estrogen receptor were generated using *in silico* docking analysis as described previously and detailed methodology is provided in supplementary information.[24-26] Docking

was based on the co-crystal structure of the naphthalene derivative GW2368 with the estrogen receptor (PDB: 3DT3).[27] Molecular docking suggests a consistent binding mode for the series of quinoline-based ERα ligands in the ligand binding domain of the human estrogen receptor. Thereby, the compounds occupy the hydrophobic cavity in a similar fashion as estradiol or GW2368 (Figure 1). The docking scores of the ERα ligands were comparable with estradiol or GW2368 (Supplementary Table 1). The quinoline scaffold of the ERα ligands occupies the position of rings A and B in the steroid and show major overlap with the naphthalene ring system of GW2368. Furthermore, presence of benzene substituents allows for interactions with His-524 in agreement with other estrogen receptor ligands. Presence of a hydrogen bond donor function is predicted to facilitate a second binding mode, where hydrogen bonds to Arg-394 and Glu-353 are formed. The structure-activity-relationship studies for the compound 4, which bearing chlorine atom at R^2 renders significant anti-proliferative activity towards ER positive cancer cells, whereas the presence of methyl group at R^1 decreases the activity. This observation was evidenced with strong binding of compound 4 to ER alpha LBD, when compared to compound 8.

In conclusion, we have identified a novel quinoline-based ERα ligands as biologically active compounds against ERα expressing human cancer cells. This study also introduces a novel microwave-assisted synthesis pathway to the compound series. Therefore, method will be useful to develop libraries of quinoline-based ERα ligands to treat breast cancer.

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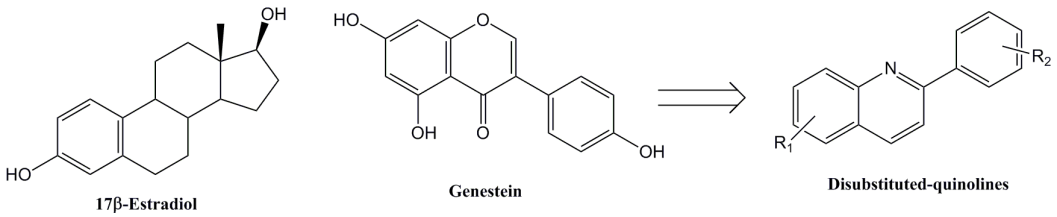
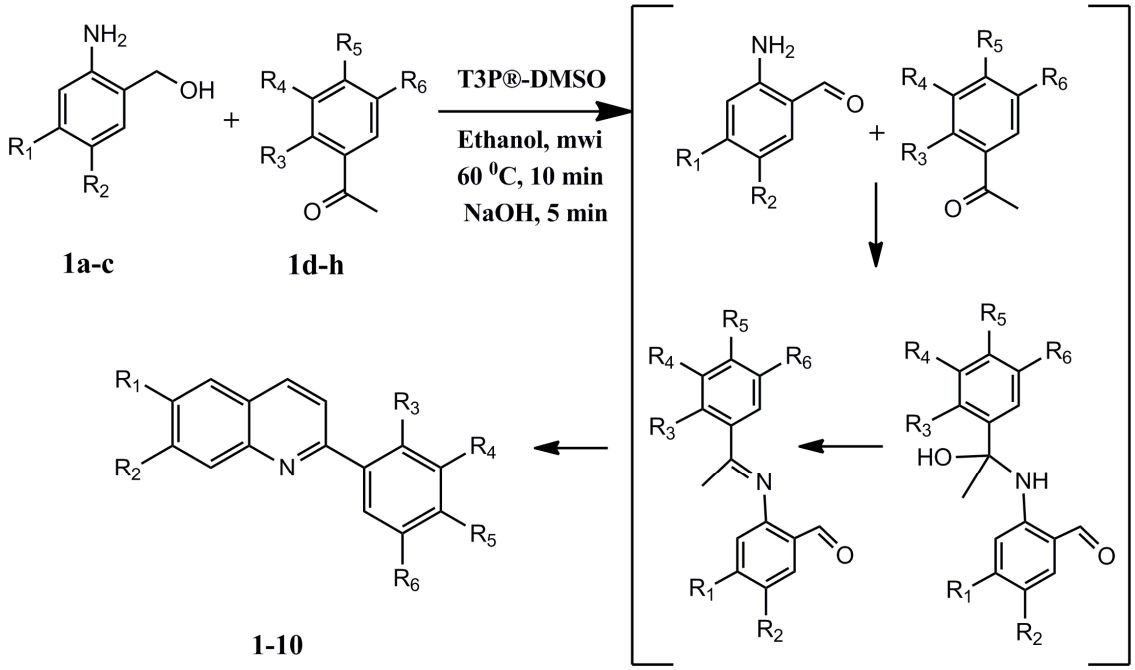
Figure Legends

Figure 1: ERα Scaffold Evolution

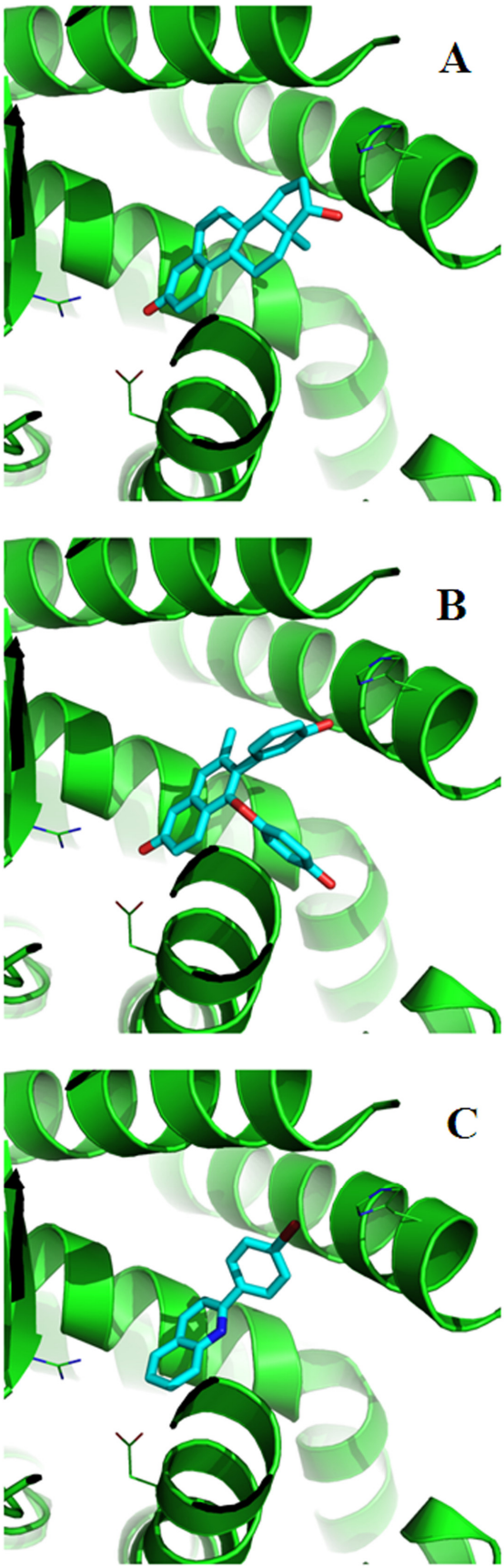
Figure 2: Schematic representation of the synthesis of quinoline-based ERα ligands.

Figure 3: Predicted interactions of quinoline-based ERα molecules towards the ligand binding domain of the human estrogen receptor. The protein is shown as green cartoon with main polar interaction centers Arg-394, Glu-353, and His-524 highlighted as sticks in atomic coloring. Interactions of estradiol with estrogen receptor (A) are resembled by the naphthalene-derived compound GW2368 (B) and predicted to be similar for the quinoline series (C).





Scheme 2



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